Such an effect may prove useful as a therapeutic manner
ic oxide production and/or biological activity is impaired.

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ial Growth Factor Upregulates the Expression of Nitric in Native and Cultured Endothelial Cells

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growth factor (VEGF) has been shown to accelerate re-endothelialization
ial thickening in balloon-injured arteries. Since endothelium-derived nitric
ular smooth muscle growth, we tested whether VEGF enhances nitric oxide
othelial cells. Experiments were performed with primary cultures of human
othelial cells (HUVEC) and with endothelium-intact rat aortic rings. Nitric oxide
pression was assessed by RT-PCR and Western blot analyses, and NOS III
tion of cGMP in HUVEC and endothelium-dependent relaxations in aortic
UVEC to VEGF165 (100 ng/ml) for 48 h led to an increase in NOS III
and in the basal level of cGMP (2.2± 0.5 fold) whereas that evoked by
(SNP) was unaffected. VEGF treatment increased in a concentration-
OS III mRNA levels within 2 h which remained elevated for the next 48 h.
t of VEGF on NOS III mRNA was abolished by protein tyrosine kinase
and erbstatin A) and was not prevented by the inhibitor of transcription
sure of aortic rings to VEGF for 7 h potentiated endothelium-dependent
nse whereas those to SNP were unaffected. Increased levels of NOS III
ere found in VEGF-treated aortic rings. These findings indicate that VEGF
expression in native and cultured endothelial cells. This effect is mediated
mase-dependent pathway(s) and seems to be due to the stabilization of
reased generation of endothelial nitric oxide may contribute to the protective
ction-induced arterial thickening.

3076

ulation of Pin, A Protein Inhibitor of Neuronal Nitric Oxide
Muscle

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synthase (nNOS) has been localized to the inner membrane of skeletal
scular dystrophy, nNOS is mislocalized to the cytosol and has been
gress of the disease. A protein inhibitor of nNOS (PIN) which specifically
vents nNOS dimerization has recently been cloned. The 89 residue PIN
regulate the biochemical activity of nNOS. We have, therefore, examined,
analysis, the distribution and regulation PIN transcript in skeletal muscles
pathological conditions where nNOS expression has been shown to be
vA was detected in limb muscles of normal rats but the highest level of
and in soleus muscle which has the lowest nNOS expression. In the
OS and PIN expressions were elevated in embryos consistent with adult
gnificant developmental regulation. In endotoxemic rats (20 mg/kg *E.coli*
OS and PIN expressions were elevated in limb muscles within 12 hours of
ve further examined the level of PIN and nNOS in the mouse myoblast cell
growing myoblast, significant PIN mRNA expression was detected. High
was maintained following the induction of myoblast fusion and in the mature
sults indicate that PIN is expressed in various skeletal muscles in-vivo and
and its expression correlates with that of nNOS. Moreover PIN mRNA
in pathological conditions such as septic shock.

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terferon Regulatory Factor-1 and Nuclear Factor κ B During S Transcription

Zaragoza, Audrey McMillan, Charles J Lowenstein, Johns Hopkins.

oxide synthase (iNOS) is expressed in a variety of inflammatory disorders in
system, and iNOS expression is regulated at the transcriptional level. We
ecular basis for the synergistic induction of the iNOS gene elicited by TNF-α
nd IFN-γ activate NF-κ B and IRF-1 in macrophages, and these transcription
tively to the κ B and the IRE sites in the iNOS promoter. To assess iNOS
how that IRF-1 and NF-κ B interact with each other. Co-immunoprecipitation
that IRF-1 and NF-κ B are bound to each other only in stimulated cells.
shift experiments show that IRF-1 and NF-κ B interact while binding to DNA
moter region. The proteins bound to a κ B site include not only NF-κ B
so IRF-1. Similarly, the proteins bound to an IRE site include not only IRF-1
These results demonstrate the existence of a physical interaction between
proteins in vivo. To explore the functional consequences of the interaction
f NF-κ B , we examined their ability to affect the structure of the iNOS
transcription factors distort promoter DNA structure at the site of binding by

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Aerosol Nitric Oxide Synthase Gene Transfer in Acute Hypoxic Pulmonary Hypertension

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Nitric oxide (NO), a vasodilator involved in the regulation of pulmonary vascular tone, is synthesized by a class of enzymes, NO synthases (NOS). We have previously shown that adenovirus-mediated overexpression of the calcium-dependent type III NOS in rat lungs reduces acute hypoxic pulmonary vasoconstriction. To quantitate the level and duration of NO production following NOS gene transfer, we measured exhaled NO by chemiluminescence in rats infected with adenovirus expressing the calcium-independent type II NOS (4 ± 10^9 pfu/ml, n=7), type III NOS (n=8), or control virus expressing no transgene (AdRR5, n=7). Exhaled NO was increased in NOS II-infected rats compared to NOS III-infected rats at 24 h (55 ppb vs 47 ppb), 4 d (52 ppb vs 36 ppb), and 7 d (31 ppb vs 22 ppb), but no longer at 10 d. The levels of NO in AdRR5-infected rats were significantly lower at all time points (19± 8 ppb at 24 h, 6± 3 ppb at 4 d and 7± 1 ppb at 7 d). To investigate whether increased pulmonary NO production after NOS II gene transfer was associated with greater inhibition of hypoxic pulmonary vasoconstriction, mean pulmonary artery pressure (PAP, mmHg) was measured during acute hypoxia (FIO_2 =0.10, 25 min) in rats 4 d after infection with NOS II (n=7), NOS III (n=8), or control virus (n=8). Acute hypoxia increased PAP from 19± 4 to 21± 5 mmHg in NOS II-infected rats compared to 23± 2 mmHg in NOS III-infected and 26± 2 mmHg in control virus-infected rats with no significant effect on systemic blood pressure. Thus, pulmonary NOS II gene transfer significantly increases pulmonary exhaled NO production for at least 7 days and is associated with a greater inhibition of acute hypoxic pulmonary vasoconstriction. Single intrapulmonary NOS II gene transfer may be a promising therapy for pulmonary hypertension.

Intercouncil Review:

Angiogenesis and Cell Proliferation:

Wednesday Morning

Convention Center Room 104A-B

Abstracts 3079 - 3088

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A Murine Model of Accelerated Diabetic Atherosclerosis: Suppression By Soluble Receptor For Advanced Glycation Endproducts

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Multiple studies suggest that lipid-independent mechanisms contribute to the development of cardiovascular disease in diabetes. Under conditions of sustained hyperglycemia, nonenzymatic glycation and oxidation of proteins and lipids results in the irreversible formation of Advanced Glycation Endproducts (AGEs) which accumulate in diabetic plasma and tissues. AGEs interact with cellular receptors such as RAGE, the Receptor for AGEs, and induce vascular cell dysfunction. The extracellular portion of RAGE (one V-type and two C-type immunoglobulin domains) is a soluble fragment (sRAGE) which we postulate may bind AGEs and block their interaction with their cell-surface receptor. We previously demonstrated a 3.7-fold increase in atherosclerotic lesion area in streptozotocin-treated apolipoprotein E deficient mice vs controls, with enhanced accumulation of AGEs and increased expression of RAGE in the vasculature by immunohistochemistry. To test if blockade of AGE-RAGE would suppress accelerated atherosclerosis, diabetic apo E deficient mice were treated for six weeks with sRAGE (20μ g/day, intraperitoneally) or equimolar mouse serum albumin (40μ g/day MSA). Mean lesion area was decreased 1.8-fold (p=0.016) in mice treated with sRAGE (150.046± 18.549 μ m^2) vs MSA (271.008± 16.721 μ m^2). No difference was observed at a low dosage of 3μ g/day; enhanced anti-atherogenic effects were observed with higher doses of 30-40 μ g/day. Serial serum glucose and HbA_{1c} levels revealed persistent hyperglycemia in both groups. There were no differences in levels of total cholesterol and triglycerides, and FPLC analysis yielded identical lipid profiles. Taken together, these data suggest that enhanced AGE-RAGE interaction likely plays a critical role in the pathogenesis of accelerated atherosclerosis in diabetes.

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Essential Role of Endothelial Nitric Oxide Synthase in Angiogenesis In Vivo

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